

1/18

Stability of HA-hGH at 37°C in cell culture media

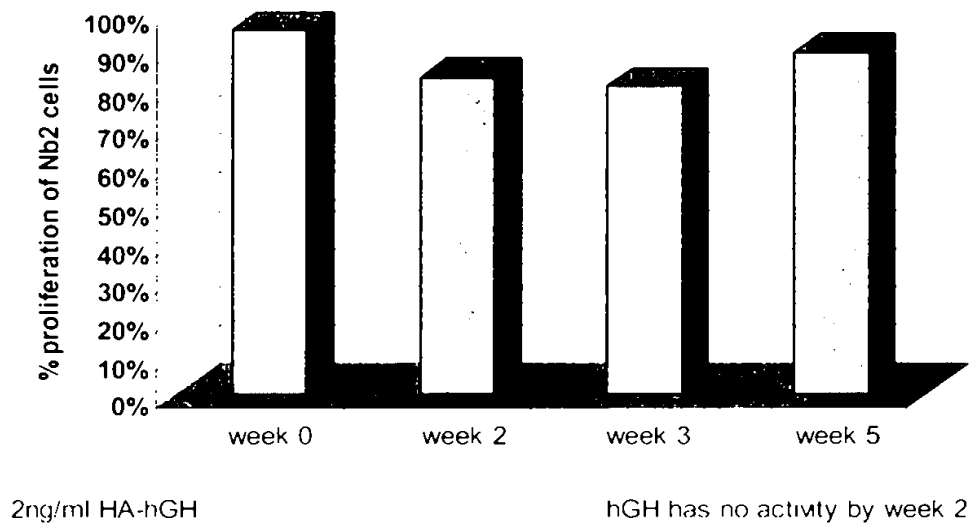


Figure 1

2/18

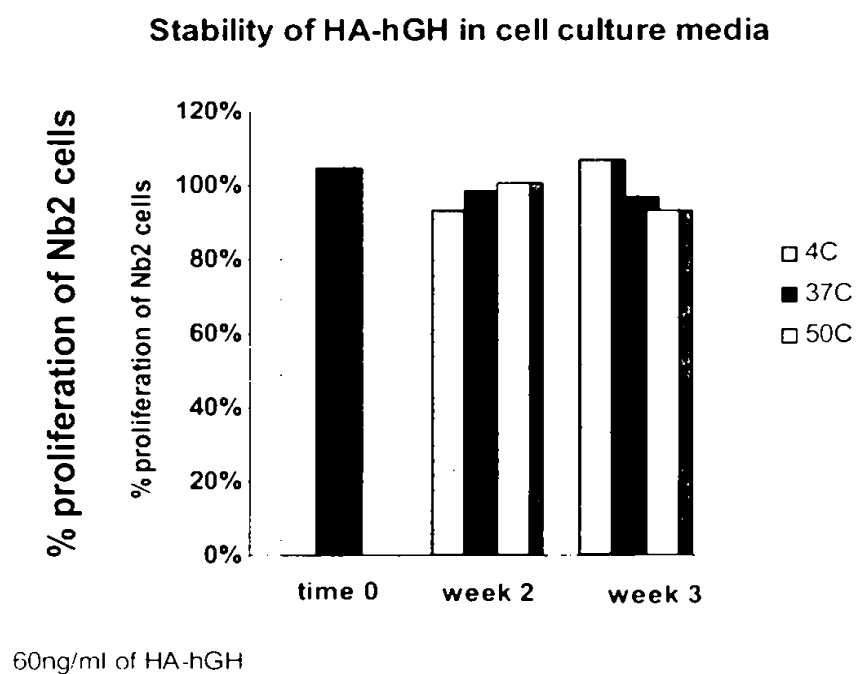


Figure 2

3/18

Nb2 Cell Proliferation Assay (24hrs)

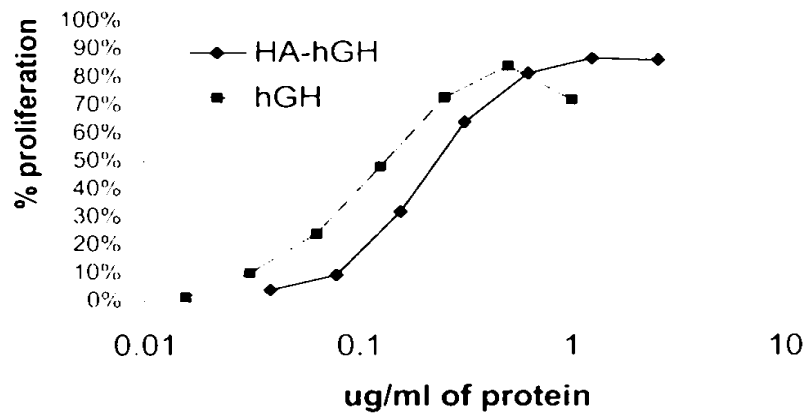


Figure 3A

Nb2 Cell Proliferation Assay (48hrs)

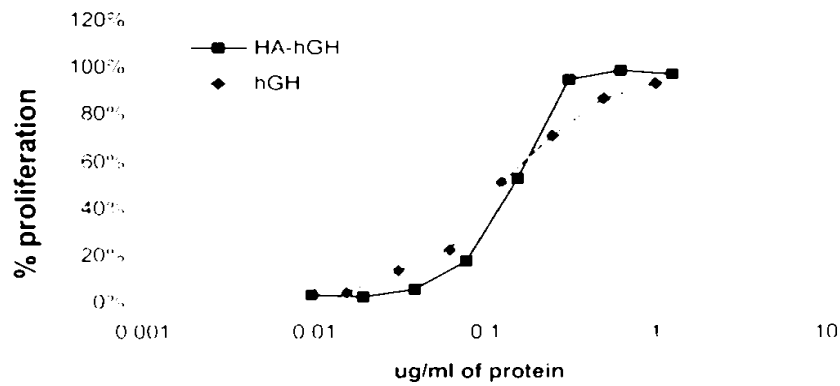


Figure 3B

4/18

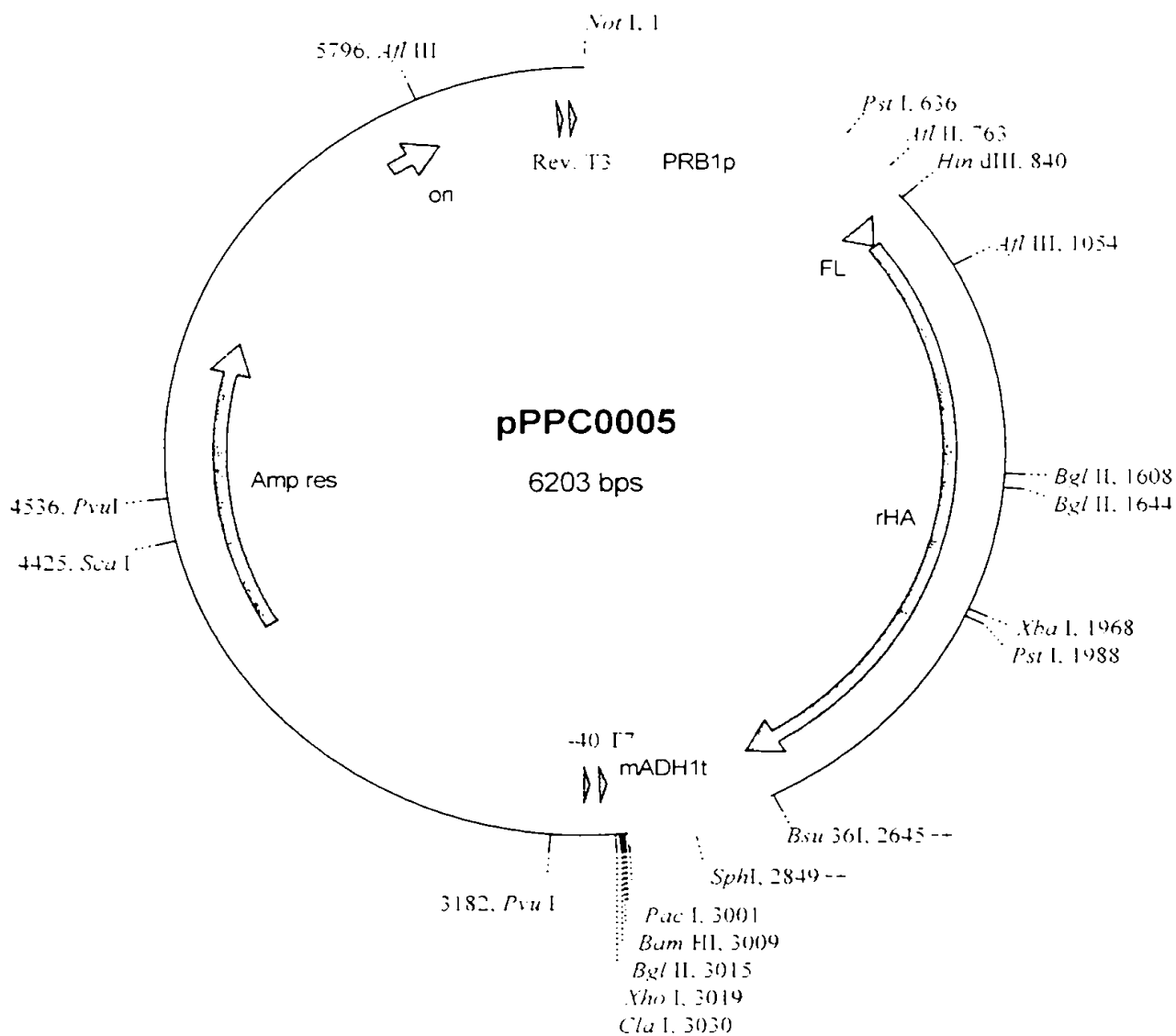


Figure 4

5/18

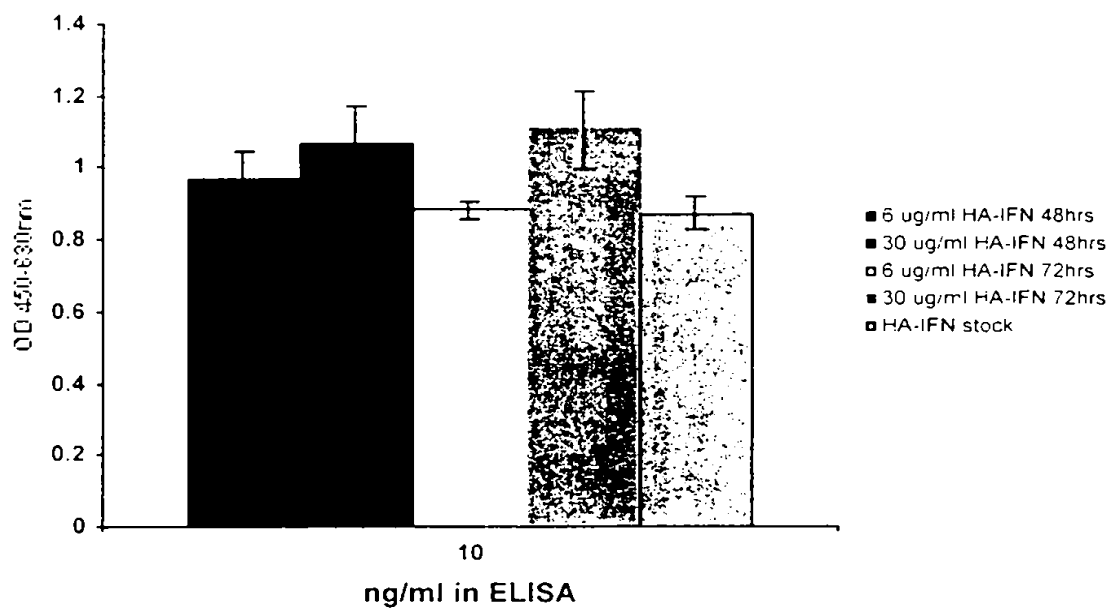


Figure 5

Figure 6

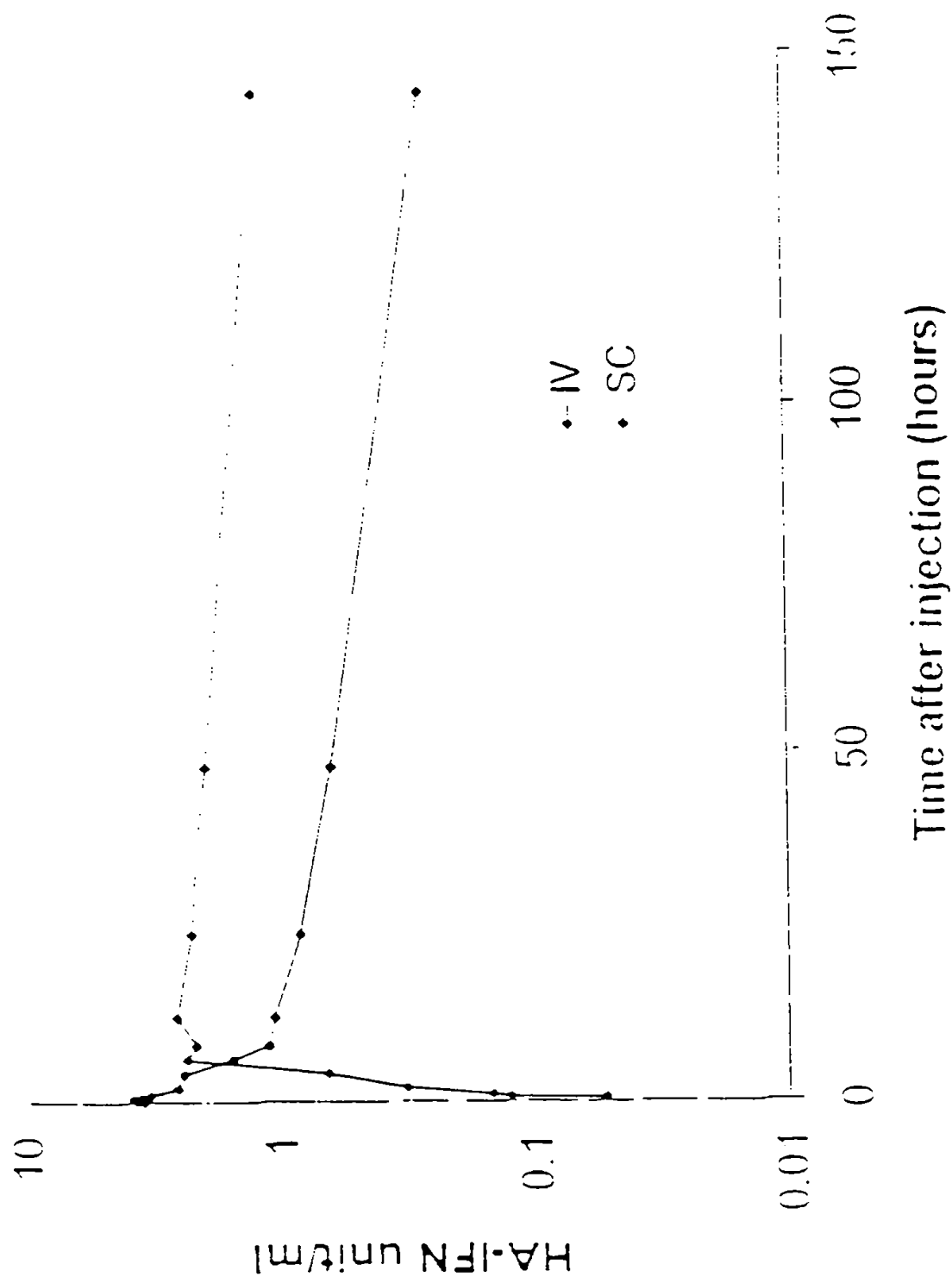
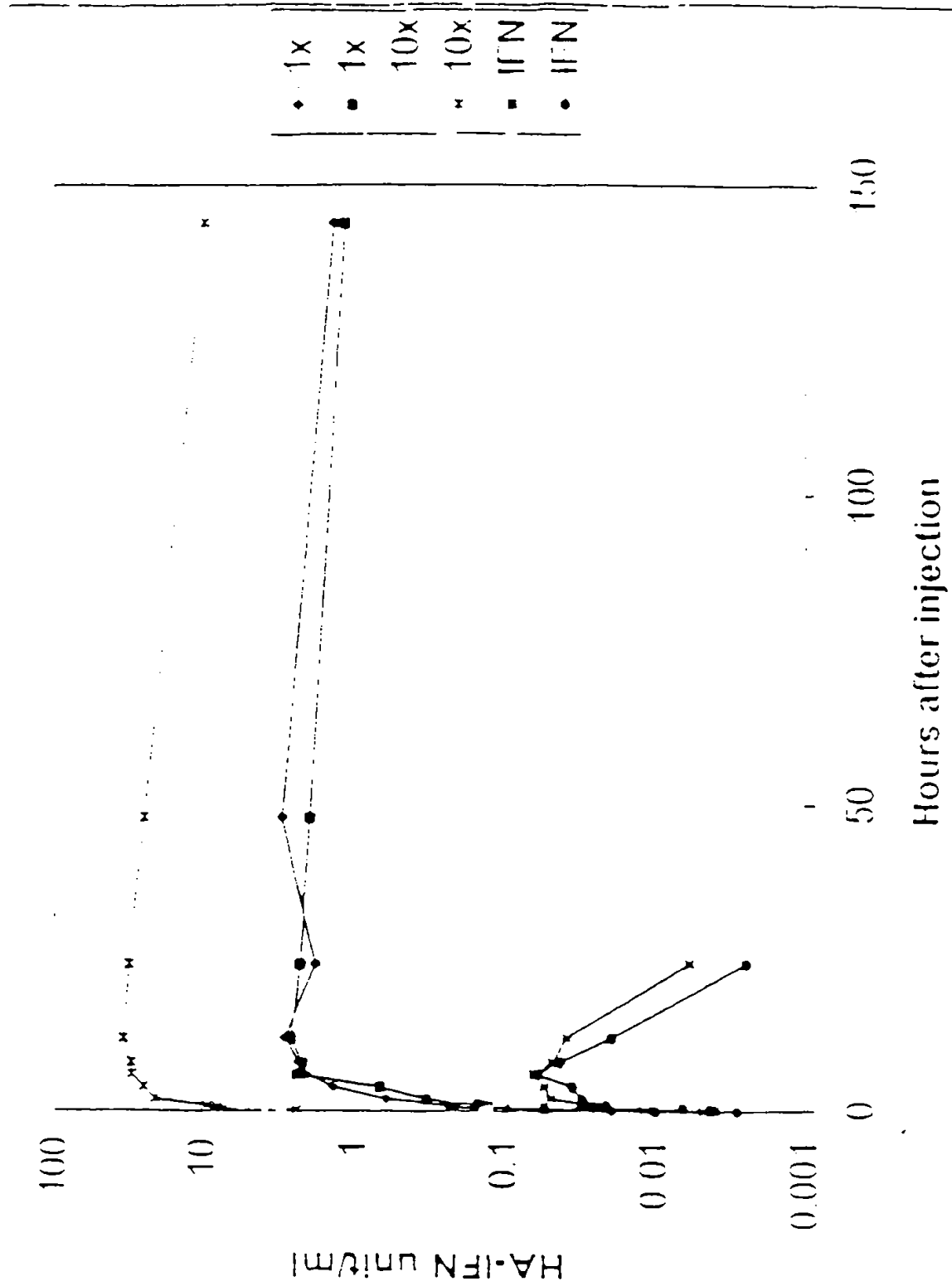


Figure 7



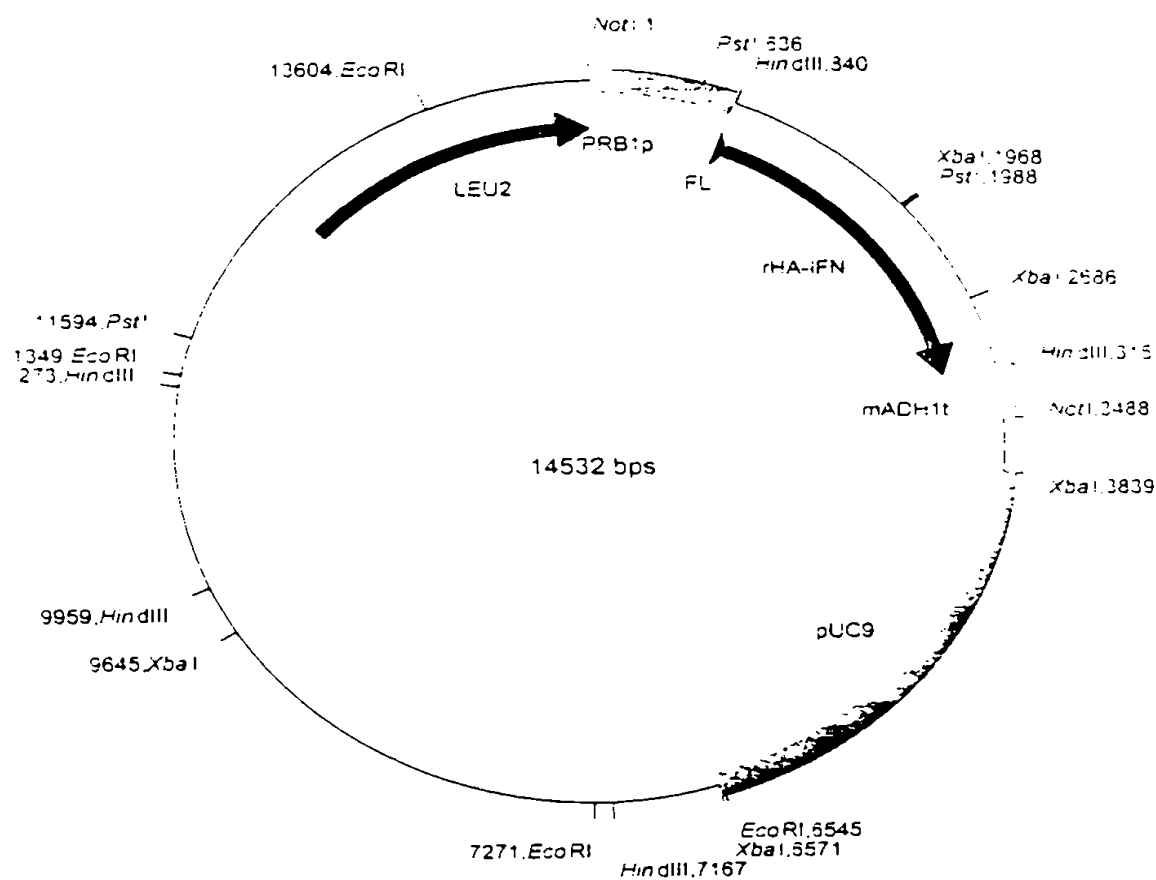


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III*/*Bam*HI fragment generally used.

Figure 8

9/18

Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

```

1  DAHKSEVAHR FKLQGEENFK ALVLIAPQY LQQCPFEDHV KL'NEVTEFA
   HHHHH HHH HHH HHHHHHHHHHH HHHHH HHHHHHHHHHH

      I                      II                      III
51  KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
   HHHHH HHHHH HHHHH HHHH H HHHH

101 CFLOHKDDNP NLPRLVRPEV DVMCTAFHDN SETFLKKYLY EIARRHPYFY
   HHHH H HHHHHHHHH HHHHHHHHH HHHHH

      IV
151 APELLFFAKR YKAAFTCCO AADKAACLLP KLDELRDGK ASSAKQRLKC
   HHHHHHHHHHH HHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

      V
201 ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTG VHTCCHGDL
   HHHHH HH HHHHHHHHHHH HH HHH HHHHHHHHHHH HHHHH HH

      VI                      VII
251 LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
   HHHHHHHHHHH HHHHH HHHHH HHHHHHH H

301 DLPSLAADFV ESKDVCKNYA EAKDVFLCMF LYEVARRHPD YSVVLLRLA
   HHHH HHHHHH HHHHHHH HHHHH HHHHHHHH

      VIII
351 KTYETTLKGC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
   HHHHHHHHHHH HH H HHHHH HHHHHHHHHHH HHHHHHH

      IX
401 YKFQNALLR YTKKVPQVST PTLVEVSRNL GKVGSKCKKH PEAKRMPCAE
   HHHHHHHHHHH HHHH H HHHHHHHHHHH HHH HHHHHHHHH

      X                      XI
451 DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPSCPSA LEVDETYVPK
   HHHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHH

501 EFNAETFTFH ADICTLSEKE RQIKKQTAUV ELVKHKPKAT KEQLKAVMOD
   HHH HHH HHHHHMEHHH HHH HHHHHHHHH

      XII
551 FAAFVEKCKK ADDKETCFAE EGKKLVAAEQ AALGL
   HHHHHHHHH HHHH HHHHHHHHHHH HH
  
```

Loop	Loop
I Val154-Asn161	VII Glu280-His288
II Thr176-Asp183	VIII Ala362-Glu369
III Ala392-Glu400	IX Lys439-Pro447
IV Gln170-Ala176	X Val440-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

```

151  APPELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

IV

```

151  APPELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

(X)_n



IV

```

151  APPELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

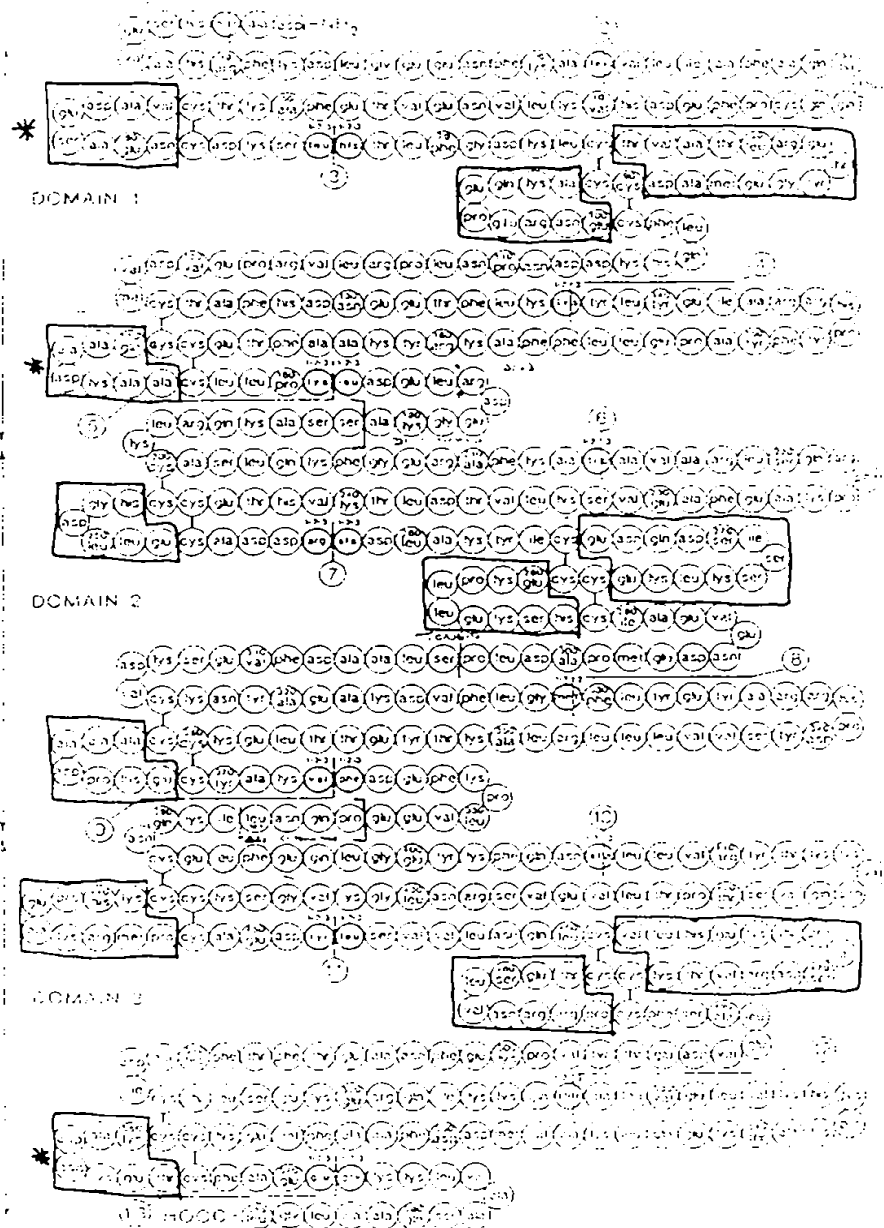
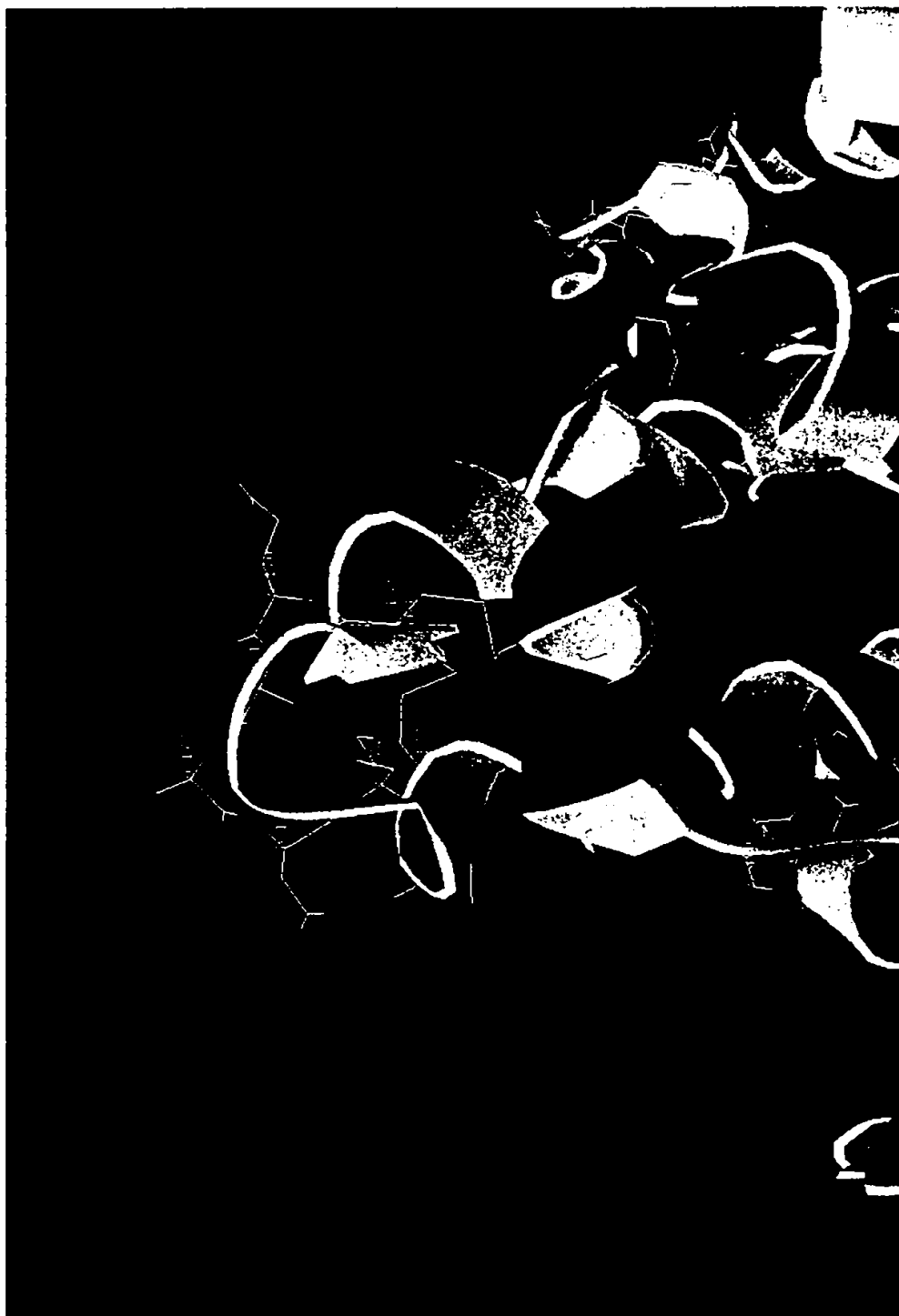


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

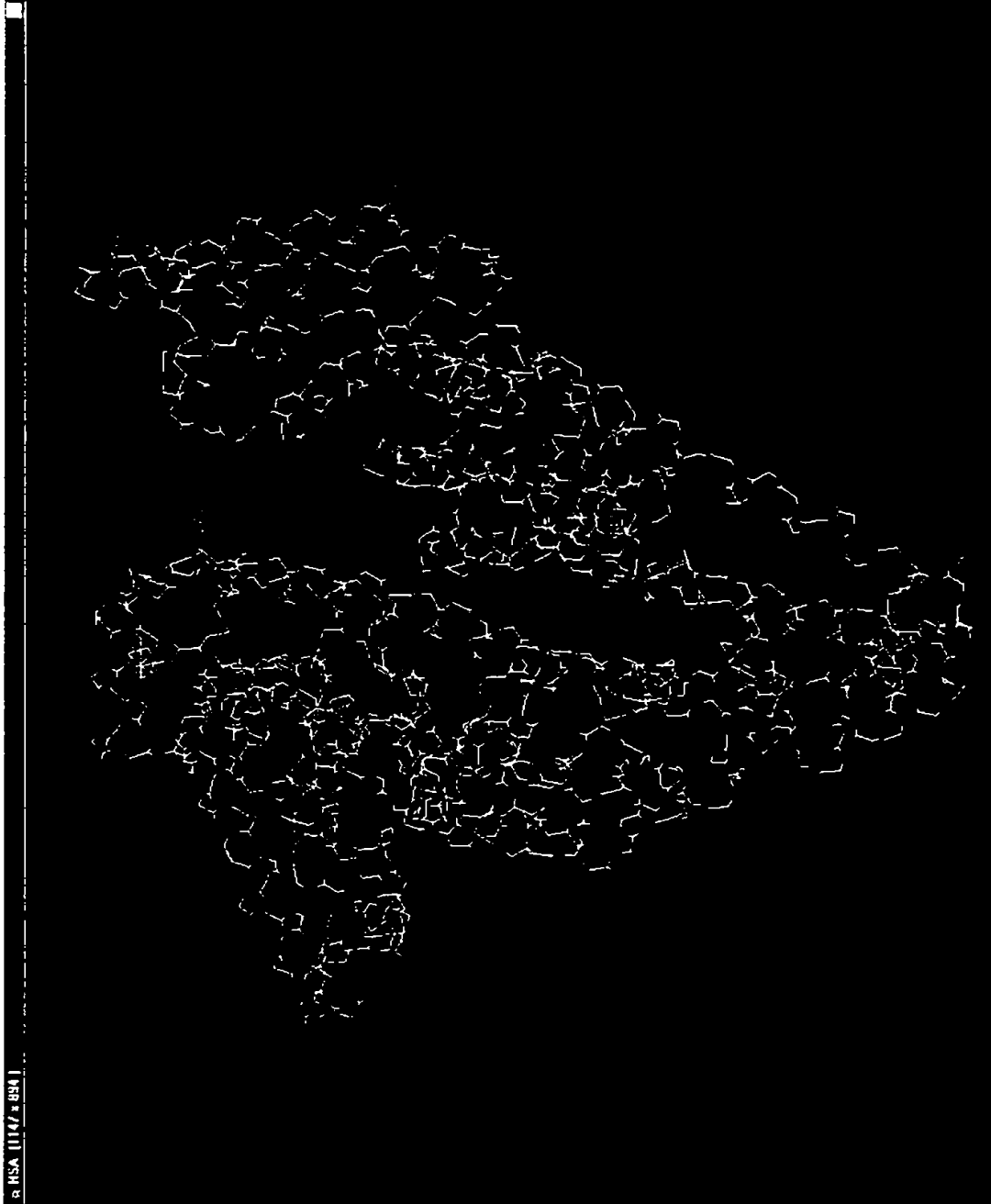


Figure 13: Tertiary Structure of HA

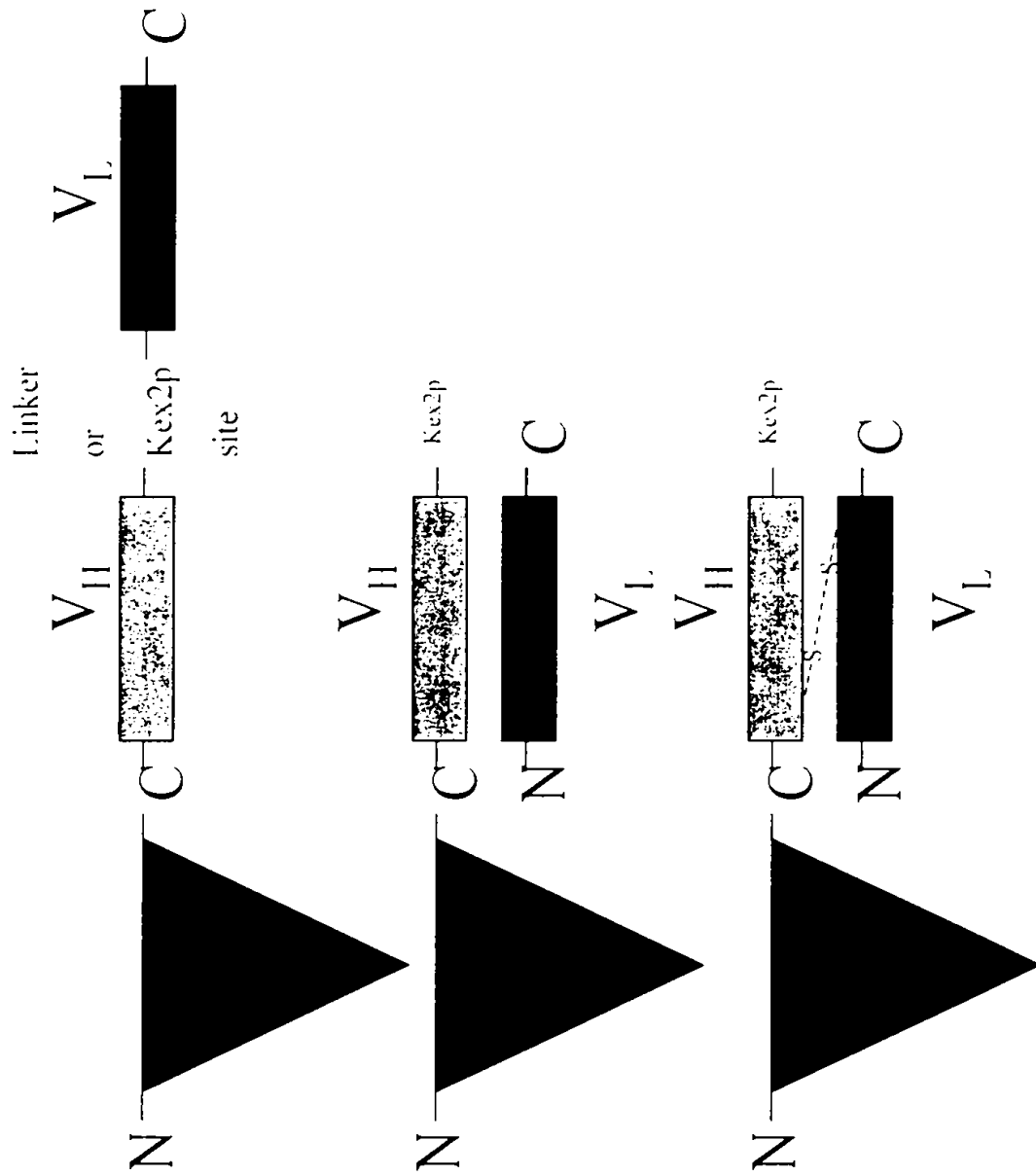


Figure 14: Schematic Diagram of Possible ScFv Fusions
 (Example is of a C-terminal fusion to HA)

15/18

1	GAT	GCA	CAC	AAG	ACT	GAG	GTT	GCT	CAT	CGG	TTT	AAA	GAT	TTG	GGA	GAA	AAT	TTC	AAA	60
10	A	H	K	S	E	V	A	H	R	F	K	D	L	G	E	E	N	F	K	20
61	GCC	TIG	GIG	FTG	ATT	GCC	TTT	GCT	CAG	TAT	CTT	CAG	CAG	TGT	CCA	TTT	GAA	GAT	CAT	120
21	A	L	V	L	I	A	F	A	Q	Y	L	Q	Q	C	P	F	E	D	H	40
121	AAA	TTA	GIG	AAT	GAA	GTA	ACT	GAA	TTT	GCA	AAA	ACA	TGT	GTT	GCT	GAT	GAG	TCA	GCT	180
41	K	L	V	N	E	V	T	E	F	A	K	T	C	V	A	D	E	S	A	60
181	AAT	TGT	GAC	AAA	TGA	CTT	CAT	ACC	CTT	TTT	GGA	GAC	AAA	TTA	TGC	ACA	GTT	GCA	ACT	240
61	N	C	D	K	S	L	H	T	L	F	G	D	K	L	C	T	V	A	T	80
241	GCT	GAA	ACC	TAT	GTT	GAA	ATG	GCT	GAC	TGC	TGT	GCA	AAA	CAA	GAA	CTT	GAG	AGA	AAT	300
81	K	E	T	Y	G	E	M	A	D	C	C	A	K	Q	E	P	E	R	H	100
301	TGC	TTG	TTG	CAA	GAC	AAA	GAT	GAC	AAC	CCA	AAC	CTC	CCC	CGA	TTG	GTG	AGA	CCA	GAG	360
101	C	F	L	Q	H	K	D	D	N	P	N	L	P	R	L	V	R	P	E	120
361	GAT	GIG	ATG	TGC	ACT	GCT	TTT	CAT	GAC	AAT	GAA	GAG	ACA	TTT	TTG	AAA	AAA	TAC	TTA	420
121	D	V	M	C	T	A	F	H	D	N	E	E	T	F	L	K	E	Y	L	140
421	GAA	ATT	GCC	AGA	AGA	GAT	CCT	TAC	TTT	TAT	GCC	CCG	GAA	CTC	CTT	TTT	GCT	AAA	AGG	480
141	E	I	A	R	P	H	P	Y	F	Y	A	P	E	L	L	F	F	A	K	160

Figure 15A

481 TAT AAA GCT GGT TTT ACA GAA TUT TGC CAA GCT GCT GAT AAA GCT GGC TGC CTG TTG CCA 540
 161 Y K A A F T E C C Q A A D K A A C L L P 180

 541 AAG CTC GAT GAA CTT CCG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
 181 K L D E L R D E G K A S S A K Q R L K C 200

 601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
 201 A S L Q E F G E R A F K A W A V A R L S 220

 661 CAG AGA TTT CCC AAA GGT GAG TTT GCA GAA GTT TTC AAG TTA GTG ACA GAT CTT ACC AAA 720
 221 Q R R F P E A E F A E V S K L V T D L T K 240

 721 CTC CAC ACG GAA TAC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AAG GCG GAC CTT 780
 241 V H T E C C H G D L L E C A D D R A D L 260

 781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
 261 A K Y I C E H N Q D S I S S K L K E C C E 280

 841 AAA CCT CTG TTT GAA AAA TAC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
 281 K P L L E E F S H C I A E V E H D E M P A 300

 901 GAG TTG CCT TTA TTA GCT GGT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
 301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E A F D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y S V V L L L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CTT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C A A A D P H E C Y A K V F D E F K P L 380

1141 CTG GAA GAG CTT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V E E P Q H L L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CTT TAC ACC AAG AAA GTA GCG CAA GTG TCA ACT 1260
 401 Y F F Q H A L L L V R Y T K F V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P T L V E V S R N L G K V G S K C C K H 440

1321 CTT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TTT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TGC 1440
 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

18/18

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1441 TTG GTG AAC AAG GGA GGA TGC TTT TGA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
481 L V N R E P P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
501 E F N A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
541 K E Q L E A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCA ACT CAA 1740
561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1762
581 A A L G L * 585

```

Figure 15D